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**Observational approaches to community structure,
from microbes to zooplankton**

Report of breakout group microbes and zooplankton

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1. Summary

Knowledge about the structure (Table 1) and dynamics of plankton communities in the ocean is essential to understanding and predicting the effects of climate change on marine ecosystems, e.g. regional variations in the draw down of CO₂ from the atmosphere, effects of ocean acidification and warming on marine biota. Phytoplankton constitutes the base of most of the marine food web and provides about 50% of the global primary production. Zooplankton forms a critical link to higher trophic levels, such as fish and cetaceans.

Plankton community structure is organized at varying levels of complexity in different ecosystems and may change seasonally and interannually in the same ecosystem. For example, the primary energy flow may occur as a short classic food chain, with few trophic groups, or as a complex food web controlled at lower trophic levels by microbial processes. Diatom blooms in coastal seas and upwelling systems constitute a major input of organic carbon to the benthic communities, while mesozooplankton faecal pellets provide a means for fast transport of organic carbon to the deep sea. Bacteria remineralise nutrients and use dissolved organic carbon, while some cyanobacteria fix nitrogen, which contributes new nutrients to the system. Many plankton organisms are mixotrophic, and viruses and other parasites infect other organisms causing disruptions in the food web.

Calcium carbonate containing organisms, such as coccolithophorids and foraminifera, have a special role in the carbonate cycle and may also be among the first organisms that are affected by ocean acidification. Harmful Algal Blooms cause ecological and socio-economic problems through fish mortalities, shellfish toxicity or hypoxia.

Table 1 Plankton size groups (based on Sieburth et al., 1978)

Name	Size range	Examples of organisms
Femtoplankton	<0.2 µm	Virus
Picoplankton	0.2-2 µm	Autotrophic prokaryotes (e.g. <i>Synechococcus</i> sp., <i>Prochlorococcus</i> sp.) Autotrophic eukaryotes (e.g. <i>Bathycoccus</i> sp.) Heterotrophic bacteria and archaea
Nanoplankton	2-20 µm	Auto- and heterotrophic flagellates, ciliates, small diatoms and dinoflagellates
Microplankton	20-200 µm	Copepod nauplii, ciliates, diatoms and dinoflagellates
Mesoplankton	0.2-2 mm	Copepods
Macroplankton	>2 mm	Copepods, krill and gelatinous plankton, e.g. jellyfish

Plankton community structure in the oceans exhibits a strong variability. It is, therefore, essential to make observations at high temporal and spatial scales to resolve this natural variability. Existing observations are often biased towards surface water due to methodological limitations. Information on plankton communities in surface waters and through the mesopelagic zone (i.e., the twilight zone) is urgently needed. In order to further our understanding of the role of planktonic communities in regional and global processes, we rely on models, which require information on both the structure (status) and the dynamics (rates) of the system. Unfortunately, technology limitations often hamper the estimation of rates *in situ*. Finally, characterization of the plankton structure requires a precise identification of species, rather than bulk measurements of the whole (or partial) biomass. We propose that the following list of prioritised biological parameters is needed for ocean observatories (in arbitrary order):

1. Biomass and abundance of phytoplankton, zooplankton (including microzooplankton), bacteria, and archaea
2. Abundance of viruses
3. Diversity of phyto-, zoo- and bacterio- and archaeaplankton as well as viruses
4. Abundance of HAB species
5. Size structure of plankton community
6. Rates, e.g. primary production, grazing, respiration, mortality, nutrient uptake/excretion
7. Simultaneous measurements of physical quantities, e.g. density structure, velocity shear and/or turbulence

It is recognised that in the foreseeable future automated systems should not replace, but be used in combination with, research vessel-based sampling and subsequent laboratory analyses (e.g. microscopy and molecular techniques). A cost efficient ocean biology observing system of plankton communities should include the following platforms:

- A. Moored systems with instrument platforms on automated vertical profilers. Single depth systems should be used only if the water column is very well mixed.
- B. Research vessels for water sampling, zooplankton net tows, use of *in situ* imaging systems and reference measurements that include optical parameters.
- C. Ships of Opportunity (SOOP) with automated instruments in flow through systems and automated water sampling.
- D. Towed instrument platforms, e.g. the Continuous Plankton Recorder (CPR).
- E. Profiling floats, e.g. further developed Argo floats
- F. Automated Underwater Vehicles (AUV)
- G. Remote sensing of ocean colour should be used together with the data from A-F

Moored ocean biology observing systems should be established together with systems for physical and chemical parameters. Sites with existing long term time series should be given priority. The often thin sub surface layers of plankton should be given special attention, since processes such as primary production and grazing are likely to be high in these layers and HAB species may proliferate there. Essential *in situ* instrumentation for observations of phyto- and microzooplankton and plankton community structure, includes imaging flow cytometers and molecular techniques, and for meso- and macrozooplankton, video systems and multi frequency echosounders. For the continuation of long time series, the continued support of the Continuous Plankton Recorder is essential. New instrumentation under development that shows great potential includes *in situ* molecular techniques and 3D holographic cameras. There is currently a gap in instrumentation for observing gelatinous zooplankton >10 cm. It is recognised that best practices for ocean biological observatories should be established and also systems for sharing near real time biological data.

2. Background and context for observing approaches related to the group's topic

Today a large part of the plankton research is confined to coastal environments and relies on ship-based sampling and measurements. Automated sampling and measurement platforms include SOOP (Ships of Opportunity), towed samplers (e.g. the Continuous Plankton Recorder), instrumented moorings, gliders and AUVs (Autonomous Underwater Vehicles). Drifting and moored vertical profilers are also part of the toolbox.

At present the main source for large scale measurements of plankton are satellites measuring ocean colour. Being based on the optical backscatter from photosynthetic pigments this approach gives important estimates of the distribution of the entire phototrophic biomass near the sea surface. However, it gives no or very little information about the phytoplankton community composition and no information about zooplankton, bacteria and viruses. In addition, phytoplankton organisms have a heterogeneous vertical distribution, with concentration maxima in sub-surface layers that are not detected from space. Thus estimates of phytoplankton biomass based on remote sensing have a relative bias towards surface concentrations. Phytoplankton subsurface layers can often be thin (centimetres to decimetres), providing concentrated prey for zooplankton.

Harmful Algal Blooms (HABs) cause impacts to the ecosystem and to human activities in coastal areas. Most HABs causing shellfish toxicity constitute only a small part of the total phytoplankton biomass and may also occur in thin layers. Remote sensing can only detect HABs occurring in surface waters and having sufficiently high biomass, which is then assumed to be homogeneously mixed. The IOC International Panel on Harmful Algal Blooms has produced a document describing recommendations for automated observation of HABs (IPHAB Task Team on HAB observations and Forecasting Systems, 2009).

At present, the study on the relevant questions concerning the future of humanity and our planet relies on hindcast, nowcast and forecast models which require high quality measurements of relevant parameters concerning both the structure and the dynamics of the ecosystems. While technology is already available to obtain structural parameters, technological limitations hamper the acquisition of the dynamical data, i.e. processes and rates. The following text will therefore mostly deal with the observation of plankton community structure. At this point, it is important to emphasise that a high level of taxonomic resolution, i.e. detailed knowledge of species composition of the community is

required (e.g. McManus et al., 2009) in order to achieve further progress in understanding the underlying processes and make reliable predictions of the future.

The plankton community structure in the oceans shows a large temporal and spatial variability. It is essential to make observations at frequencies high enough to resolve the natural variability. Point measurements at a low temporal frequency (e.g. monthly) does not provide information with adequate detail on the variability of phyto- and zooplankton biomass, biodiversity, primary production, secondary production and other important parameters. Measurements made at a too low frequency may produce artefacts (aliasing) which disguise the actual signal. Systematic long term sampling and measurements are likely to be the only way to assess effects of climate change on the marine ecosystem.

3. What are the priority observations to address this issue?

Observations should be made at appropriate temporal, horizontal and vertical resolution to resolve multiscale natural variability. Relevant physical, chemical and biological parameters should be measured simultaneously. It is recognised that in the foreseeable future automated systems should not replace, but be used in combination with research vessel based sampling and subsequent laboratory analyses (e.g. microscopy and molecular techniques).

The following platforms are suggested:

- A. Moored systems with instrument platforms on automated vertical profilers. Single depth systems should be used only if the water column is very well mixed.
- B. Research vessels for water sampling, zooplankton net tows, use of *in situ* imaging systems and reference measurements that include optical parameters.
- C. Ships of Opportunity (SOOP) with automated instruments in flow through systems and automated water sampling. The term FerryBox system is often used for these systems.
- D. Towed instrument platforms such as the Continuous Plankton Recorder (CPR).
- E. Profiling floats, e.g. further developed Argo floats.
- F. Automated Underwater Vehicles (AUV)
- G. Remote sensing of ocean colour should be used together with the data from A-F.

The following are the prioritised biological parameters:

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3. Diversity of phyto-, zoo- and bacterio- and archaeaplankton as well as viruses
4. Abundance of HAB species
5. Size structure of plankton community
6. Relevant rates, e.g. primary production, grazing and respiration, mortality, nutrient uptake and excretion
7. Physical quantities such as turbulence or shear

Table 1 Proxy for phytoplankton biomass

Observation	Depth range	Reference measurement	Unit	Comment
Vertical profiles of <i>in situ</i> chlorophyll <i>a</i> fluorescence. Profiles should have a vertical resolution of 20 cm or better	The whole water column in coastal seas 0-200 m or 0-1000 m in open ocean	Water sampling and analyses of extracted chlorophyll <i>a</i>	Chl. <i>a</i> mg m ⁻³ and Chl. <i>a</i> mg m ⁻²	Only night time profiles should be used for quantitative purposes to avoid effects of photoquenching during day time.
Multi spectral/hyper spectral optical instrumentation	The whole water column in coastal seas 0-200 m or greater in open ocean	Water sampling and HPLC-analyses of extracted photosynthetic pigments	Pigment mg m ⁻³	Method is less sensitive to day/night variation. Functional group discrimination based on pigment composition may be possible.
Integrated phytoplankton biomass of photic zone Light attenuation at 490 nm or hyperspectral (Chavez et al, 2000)	<i>Coastal seas</i> Sensors at surface and 20 m <i>Open ocean</i> Sensors at surface, 20 m and 50 m	Water sampling and analyses of extracted chlorophyll <i>a</i>	Chl. <i>a</i> mg m ⁻²	This approach is recommended if profiling systems are not available. Irradiation is measured using sensors with biofouling protection at local noon. Data is averaged for e.g. 10 minutes.
Near surface biomass covering large areas Chlorophyll <i>a</i> fluorescence measured in FerryBox system on SOOP	Ca 3-5 m	Water sampling in FerryBox systems, filtering, HPLC-analyses of extracted photosynthetic pigments	Pigment mg m ⁻³	
Near surface biomass covering large areas detected using remote sensing of ocean colour, e.g. MERIS and MODIS sensors.	Centimetres (although larger depths are often inferred if the Surface Mixed Layer (SML) is assumed homogeneous and the SML depth is known – which is often not the case)	Water sampling from research vessels and FerryBox systems, filtering, HPLC-analyses of extracted photosynthetic pigments	Chl. <i>a</i> mg m ⁻³	Data from remote sensing must be used together with data from <i>in situ</i> measurements, e.g. from profiling moorings.

Table 2 Phytoplankton (including HABs) and microzooplankton community structure

Observation	Depth range	Reference measurement	Unit	Comment
Vertical profiles using <i>in situ</i> imaging flow cytometers and SOOP with FerryBox systems	The whole water column in coastal seas 0-200 m or greater in open ocean ca 3-5 m	Regular water sampling and microscopic analysis to estimate species abundance, composition and biomass, incl. size fractionation	Species composition, organisms per litre Wet weight mg l ⁻¹ , Carbon content mg l ⁻¹ Size structure	Profiling platform needs to stop at discrete depths for 20-40 minutes for FlowCytometric analyses.
Molecular biological technique appropriate for the HAB-organism	Depends on local conditions	Regular water sampling and microscopic analysis	cells l ⁻¹	Molecular method should be verified for the local HAB species

Table 3 Meso- and macrozooplankton community structure and biomass

Observation	Depth range	Reference measurement	Unit	Comment
Optical <i>in situ</i> techniques, e.g. OPC/LOPC, UVP, VPR, SIPPER and HOLOCAM *acronyms are described below.	0-1000 m	Zooplankton net tows and optical instrumentation used from research vessel	Species composition, Dry weight/Carbon mg m ⁻³	Gelatinous zooplankton > 10 cm not always detected
Continuous Plankton Recorder	Near surface		Species composition Wet weight mg m ⁻³ Carbon content mg m ⁻³ Size structure	The CPR data is an invaluable time series but limited in depth and by selectivity
Continuous, Underway Fish Egg Sampler (CUFES)	Near surface	Ichthyoplankton net tows; optical and acoustic instrumentation from research vessel	Fish egg composition and abundance; fecundity and egg production parameters	Under use in a variety of marine systems (Western Pacific, North and South Atlantic, Mediterranean)
Acoustic techniques; multifrequency echosounders	0-1000 m	Zooplankton net tows and optical instrumentation used from research vessel	Wet weight mg m ⁻³ Carbon content mg m ⁻³ Size structure	Gelatinous zooplankton are not included, with a few exceptions (e.g., Båmstedt et al., 2003)

Table 4 Size structure of plankton

Observation	Depth range	Reference measurement	Unit	Comment
Scattering techniques such as LISST (see below) OPC/LOPC Flow Cytometry Video Multi frequency acoustics	0-1000 m	Plankton sampling and microscope analyses; Particle counter analysis; Size fractionated chlorophyll a	Size structure	The whole size spectrum of plankton is not covered by one method alone.

4. Where should the observations be made and at what frequency and duration?

Long term ocean biology observation systems should be established in all oceans and coastal seas. Figure 1 illustrates the biogeographical provinces proposed by Longhurst et al. (1995). Ideally all provinces should include replicate locations to estimate variability in each area. Initially existing ocean observation sites such as those described at www.oceansites.org and at www.ferrybox.eu should be expanded with more biological observation systems. In addition coastal systems should be established in all GOOS regional alliances such as EuroGOOS that includes BOOS (Baltic Sea) NOOS (Europe's North West Shelf). Existing locations with long term observation series should be extended with ocean biology observation systems. The systems should include both automated systems and research vessel based sampling.

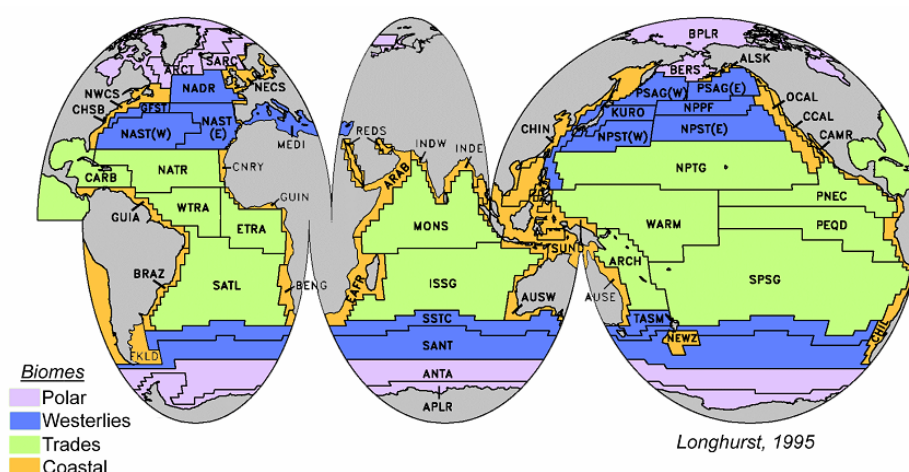


Figure 1. The structure of marine ecosystems is known to be constrained by physical forcing. This has lead to the notion of biogeochemical provinces, which relate the biological state of the marine ecosystem (e.g., community structure, productivity) to its physical environment (e.g., salinity, temperature, available light). Longhurst et al. (1995) partitioned the oceans into 57 biogeochemical provinces.

Table 5 Observation frequency

Type of observation	Recommended frequency	Duration	Comment
Automated profilers	Every 4 hours or higher	Year around	Biofouling problems are likely to limit the deployment time
Research vessel sampling	Monthly or higher	Year around	In remote locations a lower frequency is acceptable but not recommended
HAB species sampling	Depends on local conditions. Often daily sampling is necessary for predictive capability.	Year around	In some areas sampling is only carried out during harvesting of shellfish.
SOOP	Weekly in coastal seas Monthly in open ocean	Year around	The schedule of the SOOP often restricts the sampling frequency possible
CPR	Monthly	Year around	The schedule of the SOOP often restricts the sampling frequency for the CPR
Remote sensing	Daily	Year around	Clouds often constrain the use of ocean colour data. It is recommended to produce weekly composites.

5. Observational technologies now available and on the horizon, and gaps in available sensors to address the need

Biofouling is a problem when deploying instruments in the sea. After only a few days in water, optical windows are often covered with a biofilm which makes the data unusable. All *in situ* instrumentation must, therefore, be used with appropriate anti biofouling measures. The effectiveness of these measures should be verified and adjusted to local conditions. ‘

Many sensors have been developed in the last 2-3 decades for *in situ* detection of plankton organisms, but the list that follows is not intended to be exhaustive. For a detailed overview of existing instrumentation see Wiebe and Benfield (2003), Benfield et al. (2007) and Beamish and Rothschild (2009; chapters 17-20).

Optical sensors for phyto- and microzooplankton

a. *In situ flow cytometry*

A flow cytometer is a type of particle counter initially developed for use in medical science. Today instruments have been developed for use specifically in aquatic sciences. Autofluorescence and scattering properties are used to discriminate different types of phytoplankton. The different phytoplankton groups are in general not distinguished taxonomically. A standard flow cytometer is

very useful to estimate abundance of e.g. autotrophic picoplankton. A more advanced type of flow cytometer has a camera that produces images of each particle/organism. Automated image analysis makes it possible to identify organisms automatically. Manual inspection of images by an experienced phytoplankton identification specialist is required for quality control and for training the system. An example of an *in situ* system in operation today is given by Sosik and Olsen (2007) and Olsen and Sosik (2007).

b. In situ fluorometers

In situ fluorometers using blue excitation light and measuring the red fluorescence from chlorophyll are widely used to estimate phytoplankton biomass. The data should be used only as a proxy for biomass since the fluorescence may differ depending on species and physiological condition of the phytoplankton, among other factors. One important source of error that can be minimised without much effort is the photoquenching effect of sunlight. Phytoplankton actually fluoresces less when exposed to daylight, and the effect is largest near the surface. A simple solution is to measure during darkness in addition to during daytime.

In situ fluorometers with excitation and emission wavelengths suitable to detect phycocyanin and phycoerythrin are also available. These instruments have the potential to detect occurrence of certain cyanobacteria, some dinoflagellates (e.g. *Dinophysis* spp.), some cryptophytes and also the autotrophic ciliate *Myrionecta rubra* (*Mesodinium rubrum*). However, abundances of these organisms are mostly below the detection limit of these instruments at the current state of the art. Also multi wavelength fluorometers exist that aim to discriminate different groups of phytoplankton.

c. In situ scattering sensors

Scattering of light at specific wavelengths and angles is used to estimate the amount of suspended matter in the sea. Phytoplankton and microzooplankton also give a signal. Since calcium carbonate scales give a strong scattering signal these sensors are especially useful to detect coccolithophorids. An instrument with multiple scattering detectors called LISST also gives information about the size structure of particles (Karp-Boss et al., 2007).

d. In situ hyper spectral optical sensors

To utilise the information found in pigment composition of different phytoplankton hyper spectral techniques can be used. This applies both to fluorescence sensors and absorbance/attenuation sensors. Hyperspectral reflectance measurements should be included as routine measurements aboard ships or *in situ* in order to link *in situ* data to remote sensing observations of ocean colour (Kirkpatrick et al., 2000; Zielinski et al., 2009)

e. In situ imaging techniques

Imaging flow cytometers and holographic systems have been developed to image phyto- and microzooplankton; however, only relatively simple flow cytometers are commercially available.

Optical sensors for meso- and macrozooplankton

Optical sensors for the *in situ* observation of meso- and macrozooplankton can be divided into particle detection and image-forming systems (Benfield et al., 2004). Particle detectors use the interruption of a light source by zooplankton and other objects to detect, count, and measure targets as they pass through a sampling tunnel. Image-forming optics use various types of cameras to image organisms along the tow path of the instrument. Many of the optical systems allow relatively high spatial resolution of the distributions of plankton taxa and associated environmental variables. However, they seldom provide species-level identification. Some examples include:

a. LOPC/OPC

The Laser Optical Plankton Counter (LOPC) is a non-imaging instrument that provides real-time information on the size and abundance of particles by measuring the cross-sectional area of particles passing through its laser beam (Herman et al., 2004). It minimizes the problem of coincidence counting associated with the former Optical Plankton Counter (OPC) at high concentrations of small particles (Herman, 1992; Sprules et al., 1998). This instrument is commercially available and represents a robust and well-established alternative for ocean observing systems, but needs to be

calibrated for the presence of fragile particles (e.g., marine snow, large cnidarians etc.). Its predecessor OPC has been successfully applied to investigate zooplankton biomass distribution and size composition in a variety of coastal and oceanic ecosystems. A new system under development may provide images of zooplankton taxa as well.

b. VPR

The Video Plankton Recorder (VPR) is a submersible video system that can be towed through the water column to observe planktonic organisms in the size range of about 100 μm – 1 cm in an imaging volume of 117 to 750 ml at tow speeds up to 12 knots (Davis et al., 1996). In addition to towed platforms (V-fin depressor, Seasoar, and a new Fast towfish), the VPR has been deployed on ROVs (JASON and SeaRover), AUVs (REMUS), and an autonomous profiling mooring. An object feature classification system is being developed (Davis et al., 2004).

c. UVP

The Underwater Vision Profiler (UVP) is a camera system that acquires images of particles and zooplankton during a vertical profile (Gorsky et al., 2000). The UVP enumerates and measures macrozooplankton (>0.5 mm), as well as particle aggregates (>60 μm). Two video cameras (narrow angle and wide angle) image particles in 1.3 L and 10.5 L, respectively, which are recorded simultaneously at 12 Hz. This system supported an investigation of global zoogeography of fragile macrozooplankton (Stemmann et al., 2008). A new 5th generation instrument (UVP5) is a miniaturized version which simultaneously records the vertical distribution of particles and zooplankton 105 μm to 2.66 mm in length in 1.02 L imaging volume at a frequency up to 6 Hz (Picheral et al., submitted). One image is recorded every 20 cm at 1 m s^{-1} . The system has real-time image processing and post-image software (Zooprocess). The UVP5 maximum deployment depth is 3000 m. The UVP5 can also be deployed by an AUV, ROV, or moorings for either short or long term deployments.

d. SIPPER

The Shadowed Image Particle Profiler and Evaluation Recorder (SIPPER) uses a continuous imaging line scan camera and, therefore, images all particles, zooplankton, and small fish >100 μm to 10s of cm in a 96 mm depth-of-field and 96 mm width (Remsen et al., 2004). The system images 14 L s^{-1} at three knots towing speed and images are automatically identified and classified using a Plankton Image Classification and Extraction Software (PICES) (Luo et al., 2004). The SIPPER has been deployed on towed platforms and AUVs and is being adapted for profiling platforms. This system is particularly effective for studying fragile gelatinous forms, such as appendicularians, siphonophores, and doliolids, which would otherwise be destroyed or damaged in nets.

e. Holographic imaging

Submersible digital holography allows the acquisition of high resolution, three-dimensional, in-situ images of a water volume containing marine organisms (Katz and Sheng, 2009). These systems are now becoming available for *in situ* observation on abundance, size distribution and behavior of a wide range of particle sizes, from nano- to macroplankton (Pfitsch et al., 2005; Dominguez-Caballero et al., 2007). Holography has a major advantage over digital photography and video systems available to date, because it provides 3D information on organisms' orientations and their spatial arrangement in relation to each other within the volume. New compact and low power holographic imaging systems (Davis et al., 2008; Sun et al., 2008) are well-suited as biological sensors in ocean observatories, particularly for understanding the effects of 3D flow in relation to organisms dynamics, and their development and application must be encouraged.

Acoustic techniques

Bioacoustics (active transmitted sound) is a cost effective, non-destructive, and efficient technology and the only method available to assess the near-synoptic distribution of zooplankton (acoustic targets) over relatively large spatial scales (m to km) (Daly et al., 2004; Barange, 2005). Quantitative conversion of acoustic backscatter to a biological meaningful number (e.g., abundance of species) is challenging and more validation work is needed. One promising alternative is to use the acoustic signature of a “validated” species (i.e., species with well-defined acoustic properties verified by

biological samples) to train a software for further automatic species recognition (Korneliussen et al., 2009). Single frequency echosounders can be used to estimate abundance of a specific size range of organisms. Multi-frequency echosounders are required to obtain a full size spectrum. The size range is approximately a few millimetres to ca. 1 m. The continuing development of zooplankton acoustic techniques is an active area of research. Acoustic Doppler Current Profilers (ADCPs) are used to profile water velocity, but can also be used to obtain vertical distributions of relative zooplankton backscatter and to track zooplankton vertical migration. Currently ADCPs are not rigorously calibrated for biological data and, therefore, cannot be converted into abundance or biomass.

***In situ* molecular biological techniques**

Molecular techniques are used widely for plankton research in onshore laboratories today. These methods have a high potential to be used in automated *in situ* systems for investigating plankton community structure. However only a few systems exist today (e.g. Paul et al., 2007; Scholin et al., 2009), which are large and complex to operate. It is very important to verify molecular probes for identifying organisms with the local or regional species. Today most probes are specific at a detailed taxonomic level, such as species or family. There is a need for probes that are specific at higher taxonomic levels, e.g. diatoms and dinoflagellates. A method using quantitative PCR for this purpose was described by Godhe et al. (2008).

6. Storage of samples for future analyses

Molecular biological techniques are still being developed at a rapid pace. Thus it may be useful to store samples of phytoplankton (e.g. on filters) and zooplankton for future reanalyses. A suitable method is e.g. storage in liquid nitrogen or at -80°C.

Images and videos of organisms and their behaviour should be stored in a structured way and ideally be part of datasets reported to oceanographic data centres. This is a way to document the aggregated data on e.g. biomass of phytoplankton. Meta information about the sample etc. may be stored in the EXIF header of images. Future reanalysis of images and footage may reveal information not immediately available today.

7. Main gaps

Gelatinous plankton – a gap for available sensors

Gelatinous organisms > 10 cm are difficult to quantify using methods and sensors available today, although some alternatives have been explored in recent years (Båmstedt et al., 2001; Graham et al., 2003). This is a real gap since large salps, medusae, ctenophorans and siphonophorans play an important role in the ecosystem (Pauly et al., 2009). There are also indications that some species are spreading, possibly as a combined result of overfishing, eutrophication and warming of the seas (Purcell et al., 2007).

Multiple sensor packages

Today many systems are available only as separate sensors, although they are used on platforms together with other sensors. Miniaturisation and enhanced technology are likely to lead to smart sensing systems, which may have smaller space and power requirements, and interact in an adaptive sampling mode

Remote sensing

There are at least three wishes and priorities from the phytoplankton research community regarding the development of satellite remote sensing of ocean colour:

1. To increase the number of available data by deploying sensors on more satellites and possibly the use of geostationary satellites;
2. Higher horizontal resolution; and
3. Higher spectral resolution.

In addition algorithms for estimating chlorophyll in coastal seas with high amount of dissolved organic matter should be further developed. The possibility to acquire useful information on the structure of

the plankton community using satellite remote sensing is likely to be very limited. Here airborne sensors may be more useful.

Processes

As stated above, processes such as primary production and other biological rates (nutrient uptake, grazing, mortality) rates are not included above since useful automated methods are still absent. We therefore strongly encourage the development of methods and technology to fill this gap. One possibility is to further develop the *in situ* ^{14}C technique for automated use. Methods based on measurements of the photosynthesis system, e.g. fast repetition rate fluorometry, has not yet proven to be very useful in automated observing systems.

Data management

It is essential that a common practice for sharing near real time and non real time biological observation data on a global scale is established. One possibility is to build upon regional systems but it can be advantageous to build a global system that would provide data also at the regional and local level.

Best practices

Documents describing best practices (calibrations, validation of sensors, accurate metadata etc.) for biological observations are needed. The level of detail has to be high enough to ensure consistent data quality at all scales. The documents should build upon existing method descriptions used by communities of practice for plankton research. Within ICES there is a group working on zooplankton (ICES, 2006) and there is also a group working on HABs (ICES/IOC Working Group on Harmful Algal Bloom Dynamics that contributed to the recommendations for HAB observations (Anonymous, 2009). SCOR currently has a set of Working Groups tackling several aspects of plankton research, including automated systems and time-series analysis (WG 125, Global Comparisons of Zooplankton Time Series; WG 126, Role of Viruses in Marine Ecosystems; WG 130, Automatic Plankton Visual Identification).

References

- Båmstedt, U., Kaartvedt, S. and Youngbluth, M. 2003. An evaluation of acoustic and video methods to estimate the abundance and vertical distribution of jellyfish. *J. Plankton Res.* 25: 1307–1318.
- Barange, M. 2005. ICES International symposium on fisheries and plankton acoustics. Conference report. *Reviews in Fish Biology and Fisheries* 5 (4): 457–459.
- Beamish, R.J. and Rothschild, B.J. (Eds). *The Future of Fisheries Science in North America*. Springer, New York, 736 pp.
- Benfield, M.C., Schwehm, C.J., Fredericks, R.G., Squyres, G., Keenan, S.F. and Trevorrow, M.V. 2004. Measurement of zooplankton distributions with a high-resolution digital camera system. In: *Handbook of Scaling Methods in Aquatic Ecology: Measurement, Analysis, Simulation*. CRC Press.
- Benfield, M.C., Grosjean, P., Culverhouse, P., Irigoien, X., Sieracki, M.E., Lopez-Urrutia, A., Dam, H.G., Hu, Q., Davis, C.S., Hansen, A., Pilskaln, C.H., Riseman, E., Schultz, H., Utgoff, P.E. and Gorsky, G. 2007. RAPID: Research on Automated Plankton Identification. *Oceanography* 20:12–26.
- Chavez, F.P., Wright, D., Herlien, R., Kelley, M., Shane, F. and Strutton, P.G. 2000. A device for protecting moored spectroradiometers from bio-fouling. *Journal of Oceanographic and Atmospheric Technology* 17: 215–219.
- Daly, K.L., Byrne, R.H., Dickson, A.G., Gallagher, S.M., Perry, M.J. and Tivey, M.K. 2004. Chemical and biological sensors for time-series research: Current status and new directions. *Marine Technology Society Journal* 38 (2): 122–144.
- Davis, C.S., Gallagher, S.M., Marra, M., Stewart, W.K. 1996. Rapid visualization of plankton abundance and taxonomic composition using the Video Plankton Recorder. *Deep-Sea Res.* II 43: 1947–1970.
- Davis, C.S., Hu, Q., Gallagher, S. M., Tang, X. and Ashjian, C.J. 2004. Real-time observation of taxa-specific plankton distributions: an optical sampling method. *Mar. Ecol. Progr. Ser.* 284: 77–96.
- Davis, C.S. 2008. Optical imaging of ocean plankton: A fantastic voyage. In: *Digital Holography and Three-Dimensional Imaging*, OSA Technical Digest (Optical Society of America, 2008), paper DMB1, 3pp.
- Dominguez-Caballero, J.A., Loomis, N., Li, W., Hu, Q., Milgram, J., Barbastathis, G. and Davis, C. 2007. Advances in Plankton Imaging Using Digital Holography. In: *Adaptive Optics: Analysis and Methods/Computational Optical Sensing and Imaging/Information Photonics/Signal Recovery and Synthesis*. Topical Meetings on CD-ROM, OSA Technical Digest (CD) (Optical Society of America), paper DMB5.
- Godhe, A., Asplund, M.E., Hårnström, K., Saravanan, V., Tyagi, A. and Karunasagar, I. 2008. Quantification of diatom and dinoflagellate biomasses in coastal marine seawater samples by real-time PCR. *Appl. Environ. Microbiol.*, 7174–7182.
- Gorsky, G., Flood, P.R., Youngbluth, M., Picheral, M. and Grisoni, J.M. 2000. Zooplankton distribution in four western Norwegian fjords. *Estuar. Coast. Shelf Sci.* 50: 129–135.

- Graham, W.M., Martin, D.L. and Martin, J.C. 2003. In situ quantification and analysis of large jellyfish using a novel video profiler. *Mar. Ecol. Prog. Ser.* 254: 129–140.
- Herman, A.W. 1992. Design and calibration of a new optical plankton counter capable of sizing small zooplankton. *Deep Sea Res.* 39: 395–415.
- Herman, A.W., Beanlands, B. and Philipps, E.F. 2004. The next generation of Optical Plankton Counter: the Laser-OPC. *J. Plankton Res.* 26: 1135–1145.
- ICES 2006. Zooplankton monitoring results in the ICES area, summary status report 2004/2005. ICES Cooperative Research Report No. 281, 43 pp.
- IPHAB Task Team on HAB observations and Forecasting Systems 2009. IOC-IPHAB recommended procedures for automated and semi-automated HAB-monitoring and forecasting within the Global Ocean Observing System. http://www.ioc-unesco.org/hab/index.php?option=com_content&task=view&id=35&Itemid=2
- Karp-Boss, L., Azevedo, L. and Boss, E. 2007. LISST-100 measurements of phytoplankton size distribution: evaluation of the effects of cell shape. *Limnol. Oceanogr. Methods* 5: 396–406.
- Katz, J. and Sheng, J. 2010. Applications of holography in fluid mechanics and particle dynamics. *Annual Review of Fluid Mechanics* 42 (Volume publication date January 2010) (doi:10.1146/annurev-fluid-121108-145508).
- Kirkpatrick, G.J., Millie, D.F., Moline, M.A. and Schofield, O. 2000. Optical discrimination of a phytoplankton species in natural mixed populations. *Limnol. Oceanogr.* 45: 467–471.
- Korneliussen, R.J., Heggelund, Y., Eliassen, I.K. and Johansen, G.O. 2009. Acoustic species identification of schooling fish. *ICES Journal of Marine Science* 66: 1111–1118.
- Luo, T., Kramer, K., Goldgof, D., Hall, L., Samson, S., Remsen, A. and Hopkins, T. 2004. Recognizing plankton images from the Shadow Image Particle Profiling Evaluation Recorder. *IEEE Transactions on Systems, Man, and Cybernetics Part B.* 34: 1753–1762.
- McManus, G.B. and L.A. Katz. 2009. Molecular and morphological methods for identifying plankton: what makes a successful marriage? *J. Plankton Res.* 31: 1119–1129.
- Longhurst, A., Sathyendranath, S., Platt, T. and Caverhill, C. 1995. An estimate of global primary production in the ocean from satellite radiometer data. *J. Plankton Res.* 17: 1245–1271.
- Olsen, R.J. and Sosik, H.M. 2007. A submersible imaging-in-flow instrument to analyze nano- and microplankton: Imaging FlowCytobot. *Limnol. Oceanogr. Methods* 5:195–203.
- Paul, J., Scholin, C., van den Engh, G. and Perry, M.J. 2007. A sea of microbes. *In situ* instrumentation. *Oceanogr.* 2(2): 70–78.
- Pauly, D., Graham, W., Libralato, S., Morissette, L. and Deng Palomares, M. L. 2009. Jellyfish in ecosystems, online databases, and ecosystem models. *Hydrobiol.* 616: 67–85.
- Picheral, M., Guidi, L., Stemmann, L. Karl, D. Iddaoud, G. and Gorsky, G. The Underwater Vision Profiler 5: A novel instrument for high spatial resolution studies of particle size spectra and zooplankton from 0 to 3000 m. *J. Plankton Res.* submitted.
- Pfitsch, D.W., Malkiel, E., Takagi, Y., Ronzhes, Y., King, S. et al. 2005. Development of a free drifting submersible digital holographic imaging system. In *OCEANS 2005, Proc. MTS/IEEE*. New York: IEEE
- Purcell, J.E., Uye, S.-i., Lo, W.T. 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. *Mar. Ecol. Prog. Ser.* 350: 153–174.
- Remsen, A., Samson, S. and Hopkins, T. 2004. What you see is not what you catch: a comparison of concurrently collected net, optical plankton counter and Shadowed Image Particle Profiling and Evaluation Recorder data from the northeast Gulf of Mexico. *Deep Sea Res* 51:129–151.
- Scholin, C., Doucette, G., Jensen, S., Roman, B., Pargett, D., Marin II, R., Preston, C., Jones, W., Feldman, J., Everlove, C., Harris, A., Alvarado, N., Massion, E., Birch, J., Greenfield, D., Vrijenhoek, R., Mikulski, C. and Jones, K. 2009. Remote detection of marine microbes, small invertebrates, harmful algae, and biotoxins using the Environmental Sample Processor (ESP). *Oceanography* 22: 158–167.
- Sosik, H. M. and Olson, R.J. 2007. Automated taxonomic classification of phytoplankton sampled with imaging flow cytometry. *Limnol. Oceanogr. Methods* 5: 204–216.
- Sprules, W.G., Jin, E.H., Herman, A.W. and Stockwell, J.D. 1998. Calibration of an optical plankton counter for use in fresh water. *Limnol. Oceanogr.* 43: 726–733.
- Stemmann, L., Youngbluth, M., Robert, K., Hosia, A., Picheral, M., Paterson, H., Ibanez, F., Guidi, L., Lombard, F. and Gorsky, G. 2008. Global zoogeography of fragile macrozooplankton in the upper 100–1000 m inferred from the underwater video profiler. *ICES J. Mar. Sci.* 65: 433–442.
- Sun, H., Benzie, P.W., Burns, N., Hendry, D.C., Player, M.A. and Watson, J., 2008. Underwater digital holography for studies of marine plankton. *Phil. Trans. R. Soc. A*, 366 (1871):1789–1806.
- Wiebe, P.H. and Benfield, M.C. 2003. From the Hensen net toward four-dimensional oceanography. *Progress in Oceanography* 56:7–136.
- Zielinski, O., Busch, J.A., Cembella, A.D., Daly, K.L., Engelbrektsson, J., Hannides, A.K. and Schmidt, H. 2009. Detecting marine hazardous substances and organisms: sensors for pollutants, toxins, and pathogens. *Ocean Sci.* 5: 1–21.